

Table II. Determination of Hydroxylipids

Hydroxy-lipids	Amount taken, μmoles	Amount recovered (calculated from LASP curve), μmoles	Recovery %, mean	Standard deviation
Cetyl alcohol	22.54	19.5	91.47	0.89
		20.0		
		21.5		
		21.5		
		Mean 20.62		
	45.08	40.1	90.15	0.83
		39.5		
		41.4		
		41.4		
		Mean 40.60		
	67.62	60.0	89.0	0.45
		59.1		
		59.1		
		60.0		
		Mean 59.55		
12-Hydroxy steric acid	38.024	35.6	95.76	0.68
		37.5		
		36.3		
		36.3		
		Mean 36.41		
	57.036	55.5	96.18	0.41
		55.0		
		54.5		
		54.5		
		Mean 54.87		
	76.048	68.0	89.56	0.82
		67.5		
		67.5		
		69.5		
		Mean 68.12		

Table III. Determination of Added Lauryl Alcohol in Some Lipids

Lipids (vegetable oils)	Lauryl alcohol added in lipids, %	Lauryl alcohol recovered (mean of three determinations), %
Coconut oil (<i>Cocos nucifera</i>)	2.13	2.21
Peanut oil (<i>Arachis hypogaea</i>)	82.92	79.50
Linseed oil (<i>Linum usitatissimum</i>)	77.27	82.94

were determined. The values for lauryl alcohol were calculated by comparison with a calibration curve prepared with pure sodium lauryl sulfate (SLS) while the values for all other alcohols were obtained, as stated above, with reference to a calibration curve drawn with LASP. Results are recorded in Tables I and II.

Determination of Added Lauryl Alcohol in Some Lipids (Coconut, Peanut, and Linseed Oils). The seed fats employed were coconut, peanut, and linseed as solution in pyridine. The selection was made on the basis of increasing unsaturation. Thus coconut oil is a fat with a very low unsaturation (I.V. 7.7); peanut oil, a nondrying oil with a relatively high unsaturation (I.V. 90.3) and linseed oil, a drying oil with a quite high percentage of unsaturation (I.V. 181.2). In each case, a single concentration of added lauryl alcohol was determined in triplicate. Results are shown in Table III.

Application to Determination of 12-Hydroxyoleic Acid in Castor Oil. A suitable quantity of the oil, depending on the content of 12-hydroxyoleic acid as determined by pyridine-acetic anhydride method (I) was dissolved in pyridine to have

Table IV. Determination of 12-Hydroxyoleic Acid as Present in Castor Oil

Amount taken (expressed as acid) μmoles	Amount recovered (calculated from LASP curve), μmoles	Recovery %, mean	Standard deviation
35.26 ^a	33.4	96.65	0.85
	33.4		
	35.5		
	34.0		
	Mean 34.07		
52.89 ^a	51.0	95.17	0.70
	49.1		
	51.0		
	49.4		
	Mean 50.32		
17.63 ^a	15.4	88.02	0.61
	15.4		
	16.5		
	14.8		
	Mean 15.52		

^a The values were determined by the pyridine-acetic anhydride method of Ogg *et al.* (Ref. 1).

a workable solution concentration. The hydroxyoleic acid was then determined by the present photometric method. Results are presented in Table IV.

RESULTS AND DISCUSSION

Absorption Spectrum of Methylene Blue-Sulfated Alcohol Complex. The absorption spectrum of the methylene blue-sulfated alcohol complex was determined for three different concentrations of sulfated lauryl alcohol at various wave lengths from 600 μm to 680 μm in the Beckmann DU spectrophotometer. The spectra are shown in Figure 1. It is apparent from the figure that the spectra displays a bit fattened absorption maximum at 650–655 μm .

Relationship between Color and Concentration. Extrapolation of optical density values *vs.* millimicromoles of pure SLS and LASP indicates a linear relationship from a concentration of about 20 μmoles to a concentration of about 70 μmoles with respect to the hydroxylipids in a solution of 10 ml taken initially (Figure 2). Beyond this range of concentration, particularly for the alcohol sulfated and complexed with the methylene blue reagent by the present procedure, linear proportionality between color intensity, and concentration of the hydroxylipid is not assured. The molar extinction coefficient measured at 650 μm in a cell of 1 cm in length was calculated to be 9.12×10^4 .

Quantitativeness of Sulfation Reaction. The standard curves constructed with pure SLS and LASP (Figure 2) indicate a small, although not significant enough variation in slopes. The curve for pure SLS is a bit steeper than that for LASP. This might imply that the sulfation reaction probably does not represent a stoichiometrically quantitative chemical change. Nevertheless, this would not affect the results if the experimental conditions are properly adhered to, and the results are calculated with reference to a standard alcohol sulfated by the described procedure. This is confirmed by the data recorded in Tables I–IV.

Specificity of Reaction. The reaction is quite specific, or to be more precise, selective for the group of long chain hydroxylipids. No separation of the hydroxylipid itself from the nonhydroxy compound or compounds containing other reactive function(s) is needed. Employment of pyridine-sulfur trioxide complex as a sulfating agent minimizes

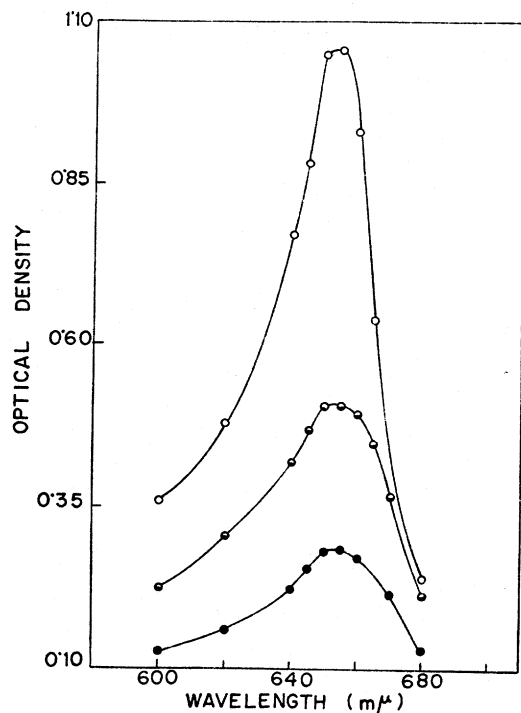


Figure 1. Absorption spectra of methylene blue-sulfated alcohol complex for different concentrations of sulfated lauryl alcohol

attack at unsaturation (16), the only function which could otherwise interfere with the reaction, and thus increases the suitability of the method.

Color Stability. Under the reaction conditions, the colored complex between the methylene blue reagent and the sulfated alcohol shows remarkable stability. No decrease in color intensity (optical density) was observed during the time interval studied (240 minutes).

Comments. The method is applicable to the determination of primary and secondary hydroxyl functions in long chain compounds. Duplication of results is premised upon strict adherence to the prescribed experimental conditions and

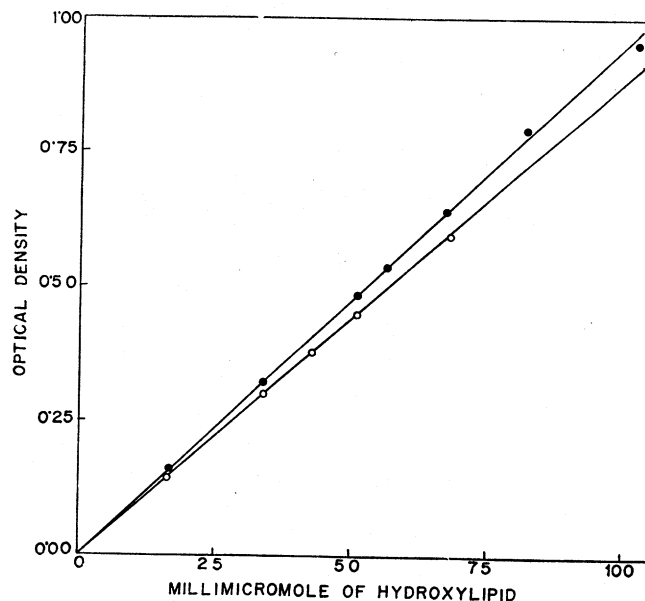


Figure 2. Relationship between optical density and concentrations of sulfated alcohols

- Pure sodium lauryl sulfate
- Lauryl alcohol sulfated by the reported method

upon maintenance of electrolyte concentrations within close limits; further the proportions of unsaturated, particularly polyunsaturated, compounds should be kept to a minimum (should not exceed 15–20% of the hydroxylipid to be analyzed).

The method is generally inapplicable to shorter chain hydroxy compounds. Apart from volatility considerations, it appears that there is a minimum molecular size requirement which should be satisfied for the complex to be formed and go into solution in the solvent phase. Application of the method to the determination of vicinal hydroxyl groups in long chain compounds such as 12,13-dihydroxy oleic acid was also not successful.

(16) A. J. Stirton, J. K. Will, A. A. Stawitzke, and S. James, *J. Amer. Oil Chem. Soc.*, **29**, 198 (1952).

3657

Photometric Method for Microdetermination of Hydroxylipids

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DETERMINATION of long chain hydroxylipids such as fatty alcohols at the sensitivity level of colorimetric analyses poses some problem, primarily because of their large molecular size and the consequent reduced reactivity and the limited solubility in the solvents normally employed for analysis. The published classical base-catalyzed acetylation and the phthalation procedures (1) for titrimetric determination of hydroxyl function are often inconsistent and not reliable for higher molecular weight alcohols, particularly at the microlevel. Various modifications and improvements of the original base-catalyzed acetylation procedure have been proposed from time to time (2-5) mostly to circumvent the difficulties arising

out of either sluggishness of the reaction rate or the reduced solubility of the samples or the necessity of using higher reaction temperatures, but none of the above procedures is suitable for determination in the microlevel. Based upon acetylation, Gutnikov and Schenk (6) in 1962 proposed a colorimetric method for microdetermination of hydroxyl function. The method utilizes the ferric hydroxamate reaction after acid-catalyzed esterification of the hydroxyl functions. This approach appears satisfactory, but the sequence of reactions involved necessarily makes this a complex procedure; further corrections have to be made for other reactive functions by colorimetric determinations before and after acetylation.

A number of other micromethods are available for analysis of hydroxyl functions. Critchfield and Hutchinson (7) suggested oxidation of secondary alcohols to ketones with acid dichromate. The ketones were then converted to 2,4-dinitro phenyl hydrazones which were measured at 480 m μ . Primary

- (1) C. L. Ogg, W. L. Porter, and C. O. Willits, *IND. ENG. CHEM. ANAL. ED.*, **17**, 394 (1945); S. Siggia, "Quantitative Organic Analysis via Functional Groups," Wiley, New York, N. Y., 1963, pp 12, 20.
- (2) J. S. Fritz and G. H. Schenk, *ANAL. CHEM.*, **31**, 1808 (1959).
- (3) R. S. Stetzel and C. F. Smullin, *ibid.*, **34**, 194 (1962).
- (4) B. D. Sully, *Analyst*, **87**, 940 (1962).
- (5) H. P. Kaufmann and E. Schmulling, *Fette, Seifen, Anstrichm.*, **64**, 319 (1962).

- (6) G. Gutnikov and G. H. Schenk, *ANAL. CHEM.*, **34**, 1316 (1962).
- (7) F. E. Critchfield and J. A. Hutchinson, *ibid.*, **32**, 862 (1960).

alcohols are oxidized to carboxylic acids and do not interfere. However, the method seems to require considerable care for good results. The reaction of 3,5-dinitrobenzoyl chloride with organic hydroxyl groups has also been utilized to provide colorimetric methods of analysis (8, 9). The procedure was tried in this laboratory but was found not to work well with high molecular weight alcohols.

An entirely new approach which does not seem to have been tried with hydroxylipids directly but which is reasonably expected to work well is the procedure involving the use of suitable dyestuff. In fact, this constitutes one of methods in use for the determination of the anionic detergent content of a surfactant solution (10). The method is based upon formation of a water-insoluble, solvent-soluble colored salt or complex of the anionic detergent and the basic dye. Rosaniline and Fuchsin have been used as the basic dye precipitating agents with chloroform or ethyl acetate-chloroform mixtures as the solvents (11). The colorimetric procedure using methylene blue has been successfully adapted to the determination of anionic detergents in sewage, where the concentrations are usually very low (12). A colorimetric method using Pontamine Fast Red 8BNL has been reported for the determination of anionic detergents in sewage (13). Thymol blue has also been recommended as a reagent for anionic detergents (14).

The problem of capitalizing on the ability of an anionic surfactant to form a water-insoluble, solvent-soluble colored complex was then to convert the hydroxyl function of the hydroxylipid into a strong anionic group capable of forming this complex. The method described here is an approach based upon this concept. The procedure involves the conversion of the -OH group to -OSO₃H group by means of pyridine sulfur trioxide complex formed *in situ* by reaction between chlorosulfonic acid and pyridine (15), formation of the colored complex with methylene blue and the sulfated compound, extraction of the complex with chloroform, and subsequent photometric estimation of the color intensity.

EXPERIMENTAL

Apparatus and Reagents. The spectrophotometer used was the Beckman DU spectrophotometer.

The glassware consisted of glass stoppered test tubes (10-ml and 15-ml capacity), 250-ml glass separating funnels, suitable pipets etc. All glassware used was of borosilicate glass and was scrupulously cleansed with cleaning powder, washed with water, rinsed with warm chromic acid, then thoroughly washed with distilled water and dried.

The reagents used were pyridine (E. Merck), purified by distillation over solid KOH; chlorosulfonic acid (BDH, 99%); chloroform (E. Merck); sodium lauryl sulfate (BDH, 99+%) *ca.* 1 μ mole/ml in pyridine; solutions in pyridine (concentration, *ca.* 1 μ mole/ml) of lauryl alcohol, cetyl alcohol, 12-hydroxystearic acid (E. Merck/Hormel Institute); and *cis*-12,13-dihydroxy oleic acid (chromatographic grade, a gift by Dr. W. E. Scott, U.S.A.) coconut

- (8) D. P. Johnson and F. E. Critchfield, *ANAL. CHEM.*, **32**, 865 (1960).
- (9) G. R. Umbreit and R. L. Houtman, *J. Pharm. Sci.*, **349** (1967).
- (10) A. M. Schwarz, J. W. Perry, and J. Berch, "Surface Active Agents and Detergents," Vol. 2, Interscience, New York, N. Y., 1958, p. 341.
- (11) G. R. Wallin, *ANAL. CHEM.*, **22**, 616 (1950).
- (12) P. N. Degens Jr., H. C. Evans, J. D. Kommer, and P. A. Winsor, *J. Appl. Chem. (London)*, **3**, 54 (1953).
- (13) G. R. Lewis and L. K. Harndon, *Sewage Ind. Wastes*, **24**, 1465 (1952).
- (14) K. Peter, *Fette, Seifen, Anstrichm.*, **56**, 997 (1954).
- (15) "Unit Processes in Organic Synthesis," 5th ed., P. H. Groggins, Ed., McGraw-Hill, New York; Kogakusha Company Ltd., Tokyo, pp 325, 327.

Table I. Determination of Lauryl Alcohol with Reference to a Calibration Curve Constructed with Pure Sodium Lauryl Sulfate

Amount taken, μ mole	Amount recovered, (calculated from SLS curve), μ mole	Recovery %, mean	Standard deviation
17.00	15.5 15.5 16.0 14.4 Mean 15.37	90.42	0.55
34.00	32.0 32.0 33.0 31.5 Mean 32.12	94.48	0.54
42.50	40.6 39.6 40.2 40.2 Mean 40.15	94.45	0.23
51.00	48.1 47.5 49.2 48.1 Mean 48.22	94.54	0.61
68.00	63.1 63.1 63.6 64.6 Mean 63.60	93.54	0.61

oil, peanut oil, linseed oil, and castor oil; methylene blue hydrochloride (E. Merck).

Preparation of Methylene Blue Reagent. Methylene blue dye, 100 mg, was dissolved in 1 liter of distilled water containing 2.5 ml of 36N H₂SO₄ and 50 grams of Na₂SO₄ (E. Merck, extra pure) and preserved in a glass-stoppered amber colored bottle in the dark.

Procedure for Analysis. An appropriate volume of the test sample was pipetted into a test tube provided with a glass stopper. The volume was made up to 2 ml with pyridine. The solution was cooled to 0 °C and 0.1 ml of chlorosulfonic acid was rapidly added to the solution along the side of the test tube. The test tube was immediately stoppered, shaken gently for a few seconds, and heated at 70 °C for 2.5 hr in an oil/glycerine bath preferably provided with a shaking arrangement. The tube was next taken out of the bath and the content quantitatively transferred into a 250-ml separating funnel by repeated washing with 55 ml of distilled water. Ten milliliters of chloroform was pipetted into the funnel, followed by addition of 5 ml of methylene blue reagent. The funnel was stoppered properly and shaken thoroughly for about 5 minutes to ensure intimate mixing of the contents and then allowed to settle till two distinct, clear layers separated out. After about 1 hr, the lower chloroform layer (which assumes greenish blue color in the presence of hydroxylipids) was separated, and the optical density of the solution measured in the spectrophotometer at 650 μ . A reagent blank without the sample was run side by side under exactly identical conditions and used to adjust the instrument to a transmittance of 100%.

All determinations reported were performed generally in quadruplicate and the results calculated with reference to a calibration curve constructed with standard lauryl alcohol sulfated by the present procedure (LASP).

Determination of Some Standard Hydroxylipids. The hydroxylipids analyzed were lauryl alcohol, cetyl alcohol, 12-hydroxy stearic acid, and 12,13-dihydroxy oleic acid. Varying concentrations of these alcohols in pyridine solution